Commercial Probiotic Products: A Call for Improved Quality Control. A Position Paper by the ESPGHAN Working Group for Probiotics and Prebiotics

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ABSTRACT

Probiotics have been proposed for a number of indications ranging from the hypothetical long-term immunomodulatory effects to proven benefits in the management of different clinical conditions. An increasing number of commercial products containing probiotics are available. In those products, irrespective if it is food, food supplement, medical food, or drug, the probiotic microorganisms have to be present in a sufficient number by the end of the shelf-life, to pass through the gastrointestinal tract resisting acid and bile, to colonize the gut, and to retain functional properties required to obtain the suggested beneficial effect. Finally, it should be contamination-free. Studies organized worldwide and summarized in this article have shown that inconsistencies and deviations from the information provided on the product label are surprisingly common. Frequently strains are misidentified and misclassified, products are occasionally contaminated, sometimes with even facultative or obligatory pathogens, strains are not viable, the labeled number of colonies cannot be verified, or the functional properties are diminished to the extent that preclude the proposed health benefit. As the probiotic preparations are commonly used for a wide range of conditions, the aim of the Working Group was to summarize results of the studies looking into the quality of the probiotic products and to raise the awareness of the important issue of their quality control. Based on the results obtained, we strongly suggest a more stringent quality control process. This process should ensure that the probiotic content as mentioned on the label meets the actual content throughout the shelf life of the product, while no contamination is present.

Key Words: children, commercial products, food supplement, probiotics, quality control, regulation

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What Is Known

- The effects of probiotics seem to be strain-specific and dose-dependent.
- Manufacturing of probiotic products can affect microbial survival, growth, and viability.
- Probiotic products are mostly categorized as food or dietary supplements, which, unlike drugs, have to comply with significantly less stringent regulatory criteria.

What Is New

- Our review provides evidence on the inadequate quality of commercial probiotic products, with regard to microorganism specification, their numbers, functional properties, and the presence of contaminating microorganisms.
- More stringent quality control procedures are suggested, which should be mandatory for products prescribed for specific clinical situations, and for use in vulnerable populations such as infants and children.

Interest for beneficial microorganisms, whose activities within fermented dairy products were recognized for centuries, resurrected in the present days and resulted in an exponential growth of probiotic preparations on the global market. Owing to increasing interest of the consumers, products containing mono- or mixed cultures of live microorganisms became an important commercial good, arriving on the markets in different forms either within the food, or in pills, sprays, liquids, suspensions, capsules, powder sachets, granulates, chewable bars, and so on. Microorganisms claimed as probiotics are being used in everyday diet for the purpose of ''improving health'' or ''to keep a healthy gut'' in otherwise fit and healthy population. In addition, probiotics are used to cure or prevent diseases in chronically ill or highly vulnerable populations like preterm infants (1–5). Consequently, a respectable number of studies was undertaken worldwide to provide valid answers. Unfortunately, most studies showing a benefit are not repeated and new studies examine new products. Therefore, the question on whether

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the quality of the preparations followed ''*hand in hand*'' the popularity of the marketed products is posed commonly in the scientific community and by health authorities.

Members of the European Society for Pediatric Gastroenterology, Hepatology and Nutrition (ESPGHAN) Working Group for Pro- and Prebiotics, who have released a number of guidelines or position paper for the clinical use of probiotics in children (6–8), are aware that they are not qualified or authorized to prepare algorithms for the manufacturing practice, or to establish and implement regulatory control mechanisms over commercially available probiotic products. The problem, however, of quality, safety, and validity of the commercial probiotic products, which are used in children, including preterm infants, prompted the ESPGHAN Working Group for Pro- and Prebiotics to perform a literature search and based on the available evidence to raise the awareness of this important issue and to provide recommendations for further actions.

METHODS

The PubMed and Cochrane Library databases were search up to June 2016. The following key terms were used: (''quality'' OR ''control'' OR ''quality control'') AND (''probiotics'' OR ''probiotic'') AND (''product'' OR ''products'' OR ''commercial''). The searches were limited to human studies and to studies published in English language. Only published data were considered. The reference lists of identified studies and key review articles, including previously published reviews, were also searched. A flow diagram documenting the identification process for each research question is presented in Figure 1.

ISSUES AFFECTING QUALITY OF THE COMMERCIAL PROBIOTIC PRODUCTS

To fulfill the definition issued by the International Scientific Association for Probiotics and Prebiotics of being *''live microorganisms that, when administered in adequate amounts, confer a health benefit on the host*'' (9), probiotics have to be present in a sufficient number within the product by the end of shelf-life, to pass through the gastrointestinal tract resisting acid and alkaline milieu, and to colonize the gut in a sufficient number required for exerting a measurable beneficial effect. Therefore, the quality of the final product depends strongly on the manufacturing processes whereby the procedures such as fermentation, matrix composition, cell harvesting, spray-drying, freeze-drying, and storage conditions like temperature, humidity, and pH are just several of a wider array of manufacturing determinants that can affect microbial survival, growth, viability, and ultimately the study results and/or clinical outcomes (10–15).

Although there are important documents conveying an opinion on a core health benefit of probiotics as a general class (9), also aligning with regulatory approaches in some countries such as Italy (16) and Canada (17), a great majority of recognized effects are strain-dependent. Numerous are the examples of indications such as prevention of nosocomial infections or antibiotic-associated diarrhea, whereby one of the well-known probiotic strains has a scientifically proven efficacy, whereas the others failed in achieving a positive result in the same setting and identical study design (6,8,18,19). Moreover, it has been described that specific properties influencing important determinants of probiotic activity such as mucosal adherence and gut colonization were restricted to the subspecies level, for example for *Bifidobacterium longum* subsp. *infantis* in comparison to *Bifidobacterium longum* subsp. longum (20). Therefore, not only the presence of a sufficient amount of live bacteria at the end of shelf life, but also the confirmed identity of the microorganism at the strain level are prerequisite requirements to ensure that a commercial product will deliver the claimed beneficial health effect.

Another emerging issue related to the product quality is the problem of substrate contamination. Contaminating microorganisms can invalidate and skew the study results making them unrepeatable in future investigations with the same probiotic strain. Much worse could be the clinical outcomes if the contaminants are facultative or obligate pathogens. A recently published case report on fatal gastrointestinal mucormycosis in a premature infant associated with a contaminated commercial dietary supplement is just one of the examples (21).

ISSUES RELATED TO TAXONOMY, NOMENCLATURE, AND CLASSIFICATION OF STRAINS

As defined in another document, the effectiveness of a probiotic product is the sum of its microbial quality and its functional properties (22). Precise identification and documentation of

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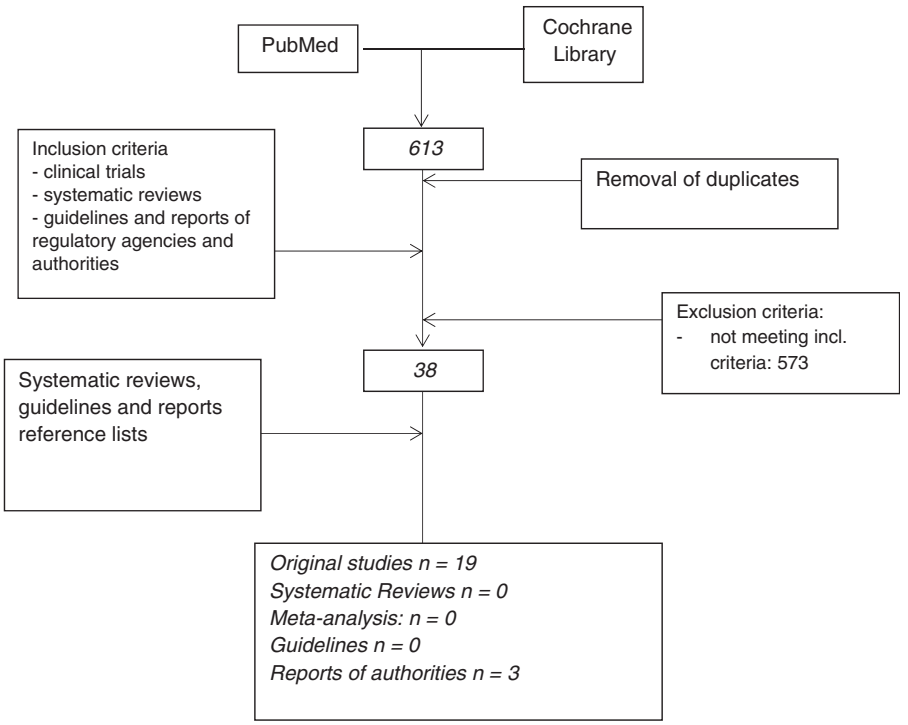

FIGURE 1. Flow chart for each research question.

both are required not only for delineation of a new ''potentially useful'' microorganism, but also for accurate identification and labeling of the already established strains in the marketed products. When creating and/or manufacturing a probiotic product, precise methodology is mandatory for each of the required steps, from typing (defined as characterization at the individual strain level), over testing of functional capacities such as resistance to acid and bile, mucosal adherence, and adhesion stability, and finally to document viability throughout the storage period. The simplest way to detect and quantify viable microbes is to look for growth of colonies on various nutrient agars, which is routinely used in all microbial laboratories. Different problems, however, could arise during cultivation such as failing to discriminate bacteria at the species and subspecies level, and in particular to distinguish viable cultivable from viable noncultivable microbes (22–24). The advantage of cultivation methods is that they will not pick up dead cells; sometimes however, especially for bifidobacteria, special growth media supplements need to be used for the correct detection and enumeration of strains. Lack of these additions may result in a falsely negative cultivation reaction (25).

Description of presently established methods, particularly those on a molecular level, is not within the scope of this document, particularly as there are many other articles covering the topic (22–24,26–32). It is important, however, to acknowledge that inappropriate identification methods are the major cause for incorrect species designations and mislabeling of probiotic products (22,33).

REGULATORY ISSUES

Regulation over probiotic products varies in respect to legal or statutory position. Although there are probiotic preparations licensed as medicinal products (pharmaceuticals/drugs), most of them are categorized as food or dietary supplements (United States, Europe), as natural health products (Canada), or as food for specific health uses (Japan) (17,34,35). In contrast to drugs that are rigorously regulated in respect to premarketing and post market safety control, including obligation for continuous monitoring, dietary supplements have to comply with significantly less stringent regulatory criteria in most parts of the world. And yet probiotics are the only group of preparations that contain live micro-organisms requiring specific manufacturing conditions to allow viable and active delivery into the correct part of the gastrointestinal tract, while retaining all the beneficial properties throughout the shelf life. Moreover, unlike other food products and drugs, there are specific safety concerns such as systemic infection, metabolic production of harmful substances, gene transfer including those responsible for antibiotic resistance, and immunomodulation, all of which are extensively covered elsewhere (22).

In the year 2006, Food and Agriculture Organization/World Health Organization has issued recommendations on the information that should be present on the probiotic product label: genus, species, and strain designation; minimum viable number of each probiotic strain at the end of the shelf life; the suggested serving size that must deliver the effective dose of probiotics related to the health claim; health claim; proper storage conditions; corporate contact details for consumer information (36). Despite the clear recommendations, however, a wide ''gray zone'' is handled by the authorities responsible for controlling the product quality, including the periodical screening of the market, and the validation of the information on the labels.

In Europe, probiotic-containing foods and food supplements are subjected to European Union (EU) regulation covered by the Food Products Directive and Regulation (37). In 2006, a novel regulation regarding all nutritional and health claims, related to all types of food, was published by the European Parliament (38). The European Food Safety Authority (EFSA) is the responsible agency in the EU for foods, food supplements, and therefore for the majority of probiotic products evaluation. More precisely, EFSA as the EU risk assessor is responsible for providing scientific advice regarding food and feed safety to support a decision-making process or setting legislation by the EU risk managers (ie, the European

Commission, the Member States, and the European Parliament). It has developed a list of safe microbial cultures defined as QPS-list (Qualified Presumption of Safety) (http://www.efsa.europa.eu/en/topics/topic/qps) designated for premarket safety assessment of the biological agents. Furthermore, EFSA is responsible for the assessment of health claims made on foods (including food supplements and probiotics) that are submitted by food manufacturers and member states. A huge number of health claims were assessed (>3000), and among them were many claims on different probiotic strains, either in more general terms such as ''boosts immune system/promotes gut health,'' or more specific regarding preventive or therapeutic efficacy in defined clinical conditions. As of October 2016, all of the claims related to probiotics were rejected (39) except for a generic claim on better lactose digestion promoted by yogurt cultures of *Lactobacillus delbrueckii* subspecies *bulgaricus* and *Streptococcus thermophilus*. Despite such rigorous and scientifically based evaluation in relation to health claims, there is insufficient control during the manufacturing process and virtually no follow-up once the probiotic product is on the market.

In the United States, probiotic products mostly fall within the Food and Drug Administration (FDA) category of dietary supplements with the granted GRAS status (generally recognized as safe), and as such are not subjected to close monitoring. This issue is extensively discussed elsewhere (34,40). Since 2007, a standardized manufacturing process is required for dietary supplements that comply with Good Manufacturing Practice guidelines issued by the FDA. These rules, however, do not address control or verification of products' quality and efficacy (41). In the United States, claims that address normal functioning known as (nonspecific) ''structure/functioning claims'' do not require governmental approval, and therefore are frequently used with probiotic products.

In summary, regulatory status of probiotic products is not established on an international basis, there is no label control, and there are no periodic screenings of the products' quality and safety.

SUMMARY OF THE RESULTS ON QUALITY ASSESSMENT OF THE COMMERCIAL PROBIOTIC PRODUCTS

Quality assessment studies are carried out worldwide with the aim to evaluate the quality of the commercial probiotic products with most coming from Europe, United States, Asia, South Africa, and Australia. Results of these studies are presented in Table 1. The major findings are summarized as follows:

Misidentification at the Genus/Species/Strain Level and Therefore Mislabeling With Regard to Incorporated Probiotic Strains (20,29,32,42–50)

Products were found to contain nonclaimed species/strains, mostly because inappropriate identification methods used. This was a common finding, in particular among the products with multiple strains, whereby some of the strains were correctly labeled, whereas the others were incorrectly designated. In one of the latest studies, aimed to determine how well label claims describe the species of detectable bifidobacteria in the product, only 1 in 16 commercial probiotic products perfectly matched its bifidobacterial label claims in all samples tested (20). There are many examples documenting that instead of claimed microorganisms with well known Generally Recognized As Safe/Qualified Presumption of Safety (GRAS/QPS) status, products were composed of potentially pathogenic genera such as *Micromonas*, *Staphylococcus*, *Enterococcus*, *Bacillus*, and so on (46,48,51).

Incongruent Numbers of Viable Cells Per Dose (29,32,43,44,46–48,52)

Many tested products contained significantly lower number of viable bacteria as compared to the numbers on the labels. A respectable number of products (up to 23%–33%) contained too few viable cells precluding the possibility of any claimed health effect (43). The viability decreased significantly over time, although still being within the declared shelf life. The quality varied between different lots, but also among pills originating from the same lot (20).

Contamination (43,45–48,53)

This is a common and particularly worrisome finding with potentially severe consequences (21).

Decreased Functional Properties (11,54,55)

Decreased acid or bile tolerance, impaired abilities to colonize and to adhere to intestinal cells, and inability to inhibit or exclude a pathogen were all found within the same species and it was influenced with the manufacturing processes and the food matrix used (11).

Conclusion

In summary, few studies yielded satisfactory results; the majority reported on >1 labeling inconsistency in most of the tested products. This finding applies for single and multistrain products, irrespective of the country of origin. Moreover, probiotic preparations licensed as medicinal products were also affected, although not to the same extent (49,51).

CONCLUDING REMARKS AND RECOMMENDATIONS

Subjects across all pediatric age groups, from birth up to transition to adult health care, are using probiotic products with increasing frequency, and are also commonly involved in the clinical studies. Furthermore, the pediatric age is particularly vulnerable with respect to safety issues, with the special emphasis on the long term outcomes. Therefore, the Working Group members address the problem and agree to provide initiatives as follows.

1. Probiotics may profoundly differ in their effects on health. Hence, precise identification of microorganisms to the strain level is required to reproduce documented effect on health.
2. Irrespective of the field addressed (research, manufacturing, quality control, and surveillance of the final product), it would be useful that probiotic products intended to improve otherwise normal diet in the healthy population are differentiated from drug-like probiotic preparations prescribed for specific clinical situations/indications. The later need to be subjected to rigorous clinical trials required for the respective application envisaged.
3. Probiotic products should be submitted to systematic quality control procedures by the respective authorities to confirm the viability and strain-level identification of the active ingredient (strain or strains). Results of these evaluations should be made public.
4. In view of the rapidly developing technology, the quality control should be performed in certified laboratories using validated and standardized methodology. Standardization and validation control should be carried out by the reference laboratories under the auspices of the respective regulatory agencies.

TABLE 1. Summary of the evidence

Author	Country	N	Type of product	Probiotic species/strain	Method of detection	Outcome	Comment
Allgeyer et al (2010) (52)	USA	10	Yoghurts	Different	Cultivation + biochemical methods	2–3 log decrease in survival in all samples during 30 days refrigerated storage period	Sensory qualities were the primary aim
Aureli et al (2010) (46)	Italy	72 samples food supplements	41 samples from 29 processing plants and 31 samples of the same brand from retailers	Different	PCR	Viability of all species reported was confirmed in 5/41 samples, 25/41 samples contained number of organisms claimed on the label; 4/41 contained species not declared (*B cereus* and *B subtilis*); 21/41 bacterial species were named with taxonomically incorrect names, 9/41 used obsolete names; after 3 mo, 7/24 samples had same composition as those from the manufacturer, after 8 mo, only 2 had same composition and after 13 months only 1 sample.	
Canganella et al (1997) (42)	Different	15 products (but 2–3 replica samples)	Pharmaceutical products	Different	Cultivation	All pharmaceutical preparations containing one single species resulted more satisfactory in terms of taxonomic definition and number of viable cells. In most of the products containing >2 species, mainly lactobacilli and bifidobacteria, these were either not found or different species were isolated.	Results presented in figures (not clearly presented)
De Vecchi et al (2008) (54)	Italy	6	Capsules, spore suspension, lyophilized preparations, oil suspension	Different	Cultivation	Assayed for storage stability, acid, base, and bile tolerance and adherence to intestinal cells. Storage at recommended condition affected *B longum*, *L casei* GG; adherence to Caco-2 cells differed, acid-base tolerance differed	
Drago et al (2010) (43)	USA	13	Different: capsules, tablets, powder	Different	Cultivation, biochemical methods	4/13 were in accordance with the package claim. 46% contained less viable, 38% lower number of species than declared, 54% were contaminated	
Fasoli et al (2003) (44)	Italy	14	7 Probiotic yoghurts and 7 lyophilized preparations + plain yoghurt as a control	Different	PCR	Lower number of CFU in majority of products (2/7 products had <10 CFU); the only 2 species of *Bifidobacterium* found in the tested products were *B breve* and *B lactis*. 1 Product contained *B subtilis* and 1 product contained *Staphylococcus*	Testing method was the primary aim
Goldstein et al (2014) (56)	USA	5	Pharmaceuticals	Different	Cultivation	Only 1/5 products did not state an expected concentration	Only study with beneficial findings (only 5 cases)

TABLE 1. (*Continued*)

Author	Country	N	Type of product	Probiotic species/strain	Method of detection	Outcome	Comment
Grzeskowiak et al (2011) (11)	Finland (origin of products from different countries)	13	2 capsules, 2 infant formulae, 3 freeze-dried powders, 4 from soft agar	*Lactobacillus rhamnosus GG*	16S rRNA gene analysis, DNA-PCR	All tested isolates were of confirmed labeled identity, all had similar acid tolerance, pathogen exclusion properties differed significantly depending on the probiotic product	
Hamilton-Miller et al (1996) (47)	Britain	13	Lyophilized prep. in capsules (10), powder (1), tablet (2)	Different	Cultivation	Only 2/13 products matched label qualitatively and quantitatively. Other missed some species, contained contaminants, or number of viable units were less than tenth of those stated	
Hamilton-Miller et al (1999) (51)	Britain	52 (44 from U, 8 from EU countries)	Yoghurts, health products, probiotic supplements	Different	Cultivation	Most products did not match label: only 4/52 contained claimed species, most had decreased numbers. In 9/52, *Enterococcus faecium* was found. Products sold for medicinal purpose had better quality	
Huys et al (2006) (30)	Different	26 companies provided strains—213 bacterial cultures	213 cultures of bacteria intended for probiotic, nutritional, research use	Lactic acid bacteria and propionibacteria	FAFLP and (rep)-PCR fingerprinting	In 85%, the species name as supplied by the depositor was confirmed; the probiotic strain category included more strains with incorrect species designations (28.1%) than did the nutritional (11.4%) and research (14.0%) strains	
Lewis et al (2016) (20)	USA	16	Not defined	Bifidobacteria	DNA-based methods	1/16 tested product matched completely label claims in all samples tested, most contained nonbifidobacterial species, most products did not contain labeled species	Unclear presentation of results
Marcobal et al (2008) (48)	USA	14	Lyophilized preparations in tablets and capsules	Different	T-RFLP, PCR	Only 1/14 contained exact species stated on the label; 7/14 had microbial contaminants; 5/14 was missing 1 claimed species	Assessment of the T-RFLP
Masco et al (2005) (29)	Belgium	58	22 yoghurts, 5 diary fruit drinks, 28 food supplements, 3 pharmaceuticals	Bidifobacteria	Cultivation, Box-PCR PFGE	Bifidobacteria could not be isolated from 51.6% of freeze-dried products. Almost all yoghurts had viable bacteria, high percentage of incorrectly labeled with respect to strain identity	
Szajewska et al (2004) (49)	Poland	5	Products licensed for medicinal purposes	Lactobacilli, bifidobacteria	Cultivation, 16S rRNA, PCR	3/5 contained strain claimed on the label; no contamination detected; 89% contained counts claimed on the label	Medicinal products!
Temmerman et al (2003) 1 (53)	8 European countries	55 Products	30 dried food supplements + 25 dairy products	Different	Cultivation	In dairy products—10^5–10^9 CFU/mL; in food supplements <1–10^6 CFU; viable bacteria could not be isolated from 11/30 food supplements; only 6 products yield all species indicated; in 19 products isolated species were entirely different from mentioned on the label	

TABLE 1. (*Continued*)

Author	Country	N	Type of product	Probiotic species/strain	Method of detection	Outcome	Comment
Theunissen et al (2005) (45)	South Africa	20	11 yoghurts 8 lyophilized preparations, 1 baby formula	Different	PCR-based DGGE analysis	54.5% of probiotic yoghurts contained labeled micro-org. vs only 33.3% of lyophilized products	Testing the method was the aim
Vanhee et al (2010) (55)	Belgium	15	Different pharmaceuticals	*Saccharomyces boulardii*	Microsatellite typing + SPC	Identity confirmed in all products; <1% of *S boulardii* survived 120 min in gastric conditions and storage for 3 mo at 40°C with 75% humidity	
Morovic et al (2016) (50)	USA	52	Different commercial products of different retailers	Different	Flow cytometry, mPCR, HTS of 16S rRNA,	33% of sample were significantly below label claim; 42% of products with incorrect taxonomy, missing species, containing unlabeled species	

CFU = colony forming units; DGGE = denaturing gradient gel electrophoresis; FAFLP = fluorescent amplified fragment length polymorphism; HTS = high-throughput sequencing; mPCR = multiplex PCR; PCR = polymerase chain reaction; PFGE = pulsed-field gel electrophoresis; SPC = solid-phase cytometry; T-RFLP = terminal restriction fragment length polymorphism.

5. Recommendations 3 and 4 should ideally apply to all probiotic-containing products, but are mandatory for products intended for use in vulnerable populations such as neonates (preterm and term), infants, and children, or in defined clinical conditions or if marketed as pharmaceutical products.
6. Adverse events, potentially related to probiotic products, should be reported and a register of those events should be maintained by health authorities.

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